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THE EFFECTS OF PROGRESSIVE RESISTANCE TRAINING ON SKELETAL
MUSCLE PROTEIN DEGRADATION IN MAN

by

Rudolph Gino Villani

A Thesis
submitted to the Faculty of Graduate Studies
through the Faculty of
Human Kinetics in Partial Fulfillment
of the requirements for the Degree
of Master of Human Kinetics at
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Windsor, Ontario, Canada
1983

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ABSTRACT

THE EFFECTS OF PROGRESSIVE RESISTANCE TRAINING ON SKELETAL MUSCLE PROTEIN DEGRADATION IN MAN

by

Rudolph Gino Villani

The influence of resistance training on human skeletal muscle protein degradation has been estimated by measurement of the urinary excretion of 3-methylhistidine (3-mehis). Sixteen male subjects on a meat-free diet were randomly assigned to control (C) and experimental (E) groups. Pre-training strength tests indicated no strength difference between the groups ($P > .05$). However, following three weeks of daily one hour weight training sessions, the E group increased its strength index by 18% ($P < .05$). Twenty-four hour urine samples were collected for all subjects at pre-selected intervals throughout the experimental period. These samples were analysed for total creatinine (Cr) mmol/day, urea (U) mmol/day, and 3-mehis umol/day. As was anticipated from previous studies, the Cr excretion remained stable throughout the training period (C = 13.88 SE. 1.09 mmol/day, E = 14.9 SE. 0.79 mmol/day). In contrast, the 3-mehis/Cr ratio, an indicator of contractile protein degradation, decreased ($P < .05$) from a baseline level of 17.97 SE. 1.31 umol 3-mehis/mmol Cr/day to 14.78 SE. 1.37 umol 3-mehis/mmol Cr

/day during the training period for the E group; this ratio remained unchanged for the C group. The maximum decrease in the 3-mehis/Cr ratio (18%) occurred after only two days of training. Thereafter, up to the final day of training, this ratio increased by 4-10% but was still significantly ($P < .001$) lower than that of the C group. Within one day of the cessation of training, the 3-mehis/Cr ratio increased significantly ($P < .05$), indicating that a quick increase in the degradation rate occurred with the removal of the progressive resistance training stimulus. Hence, this study would suggest that a process of protein conservation takes place very rapidly in response to a resistance training stimulus, as a result of decreases in muscular proteolysis.

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INTRODUCTION

It is now acknowledged that progressive resistance training increases the myofibrillar protein content of skeletal muscle. However, there is little information on the mechanisms responsible for this enhanced deposition of intracellular protein. It is known (44) that the content of any intracellular protein is dependent on the balance between its rate of synthesis (K_s) and its rate of degradation (K_d). Therefore, in theory the enhanced storage of myofibrillar protein may occur either by an increase in its synthetic rate or by a decrease in its proteolytic rate or by the inter-action of both. Several investigators have suggested that the myofibrillar synthetic rate is elevated when skeletal muscle is repeatedly forced to contract against an overload (5, 11, 12, 26, 27, 46). An enhanced protein synthesis rate has been suggested because of the observed increase in: amino-acid transport across muscle cells (5, 12), incorporation of labelled amino-acids into muscle proteins (11, 26, 27), and metabolism and content of nucleic acids (11, 46) in the working muscle cells. In contrast, knowledge of the contribution of myofibrillar proteolysis to work-induced muscle growth is less clear. This uncertainty reflects upon the overall lack of information about, a) the physiological significance of this process b) the factors regulating in vivo protein degradation, c) the biochemical mechanisms of the degradative process, d) the most accurate ways of measuring in vivo degradative rates.

THE PHYSIOLOGICAL SIGNIFICANCE OF PROTEIN DEGRADATION

Continuous degradation of cellular proteins to their constituent amino-acids may seem wasteful. However, there are several benefits which can be gained by the organism from this process. It has been suggested (10) that proteolysis enables the organism to dispose of harmful mistakenly synthesized proteins, which have occurred from errors in gene expression (mutations) and chemical modification or denaturation of normal proteins. In addition, continuous degradation and new synthesis (protein turnover) augments the organisms chances of adapting readily to environmental stimuli. For instance, exercise patterns influence both the size of muscle fibre types (17, 25), and the types of enzymes stored within muscle tissue (25). Indeed, it has been determined (43) that the more rapidly an enzyme is degraded, the faster its intracellular content can alter in response to, hormonal, nutritional, and environmental stimuli. Finally, muscular proteolysis provides the organism with a large energy bank which can be borrowed from in times of decreased caloric intake. It has been demonstrated that during the initial stages of starvation, muscle degradation increases and the amino-acids produced are utilized to provide the organism with necessary glucose (32).

BIOCHEMICAL MECHANISMS OF THE DEGRADATIVE PROCESS

While many of the biochemical mechanisms of the protein synthesis process are known, proteolytic mechanisms are less well understood.

Much of the current knowledge concerning proteolysis has been gained from the study of its regulatory system. From the results of several studies (9, 13, 26), two intracellular proteolytic pathways have been proposed. One proteolytic pathway was thought to be equated with autophagocytosis and regulated by several in vivo factors, while a second pathway is believed to be unregulated and responsible for the degradation of unstable (mutations) proteins.

The former pathway is dependent on the degradation or hydrolysis of proteins by a host of enzymes located in the cellular organelles called lysosomes. Although the role of lysosomes as the intracellular site for the catabolism of exogenous proteins taken into the cell is clearly established, the lysosomal role for the breakdown of endogenous proteins is much less definite (3). For hepatic tissue, there is evidence which suggests that the hormones regulating intracellular protein breakdown also regulate the number of intracellular lysosomes. For example, insulin is known to decrease hepatic proteolysis while it simultaneously reduces intracellular lysosome count (34, 37).

Protein breakdown pathways of a non-lysosomal nature are more common for the proteolysis of unstable intracellular proteins. Instability of protein may theoretically arise if the primary sequence of the protein is altered by a mutation, insertion of abnormal amino-acids, or a post translational modification (3). When mutations caused the production of incomplete fragments of the enzyme beta-galactosidase, in E-coli bacteria, these fragments were more readily degraded by proteases than complete molecules (14).

2

4

VALIDATION OF URINARY 3-METHYLHISTIDINE AS A MEASURE OF PROTEIN DEGRADATION

The first demonstration of protein turnover in animals (44) was made by measuring the rates of incorporation of 15-N labelled amino-acids into protein and the rates of loss of this label from protein. Since this work, several variations of Schoenheimer's (44) method have been used to estimate proteolysis. The most common isotopic technique still in use is the pulse-labelling approach, in which a cell or organism is administered a radioactive precursor to label cell protein. After the protein is purified, its radioactivity is assayed as a function of time. A decrease in the specific activity of the protein is taken as a measure of the proteolytic rate, on the assumption that the new proteins being synthesized are non-radioactive (10). However, this assumption has been invalidated, since labelled amino-acids released by proteolysis can be reutilized in another round of protein synthesis. For the organism, this recycling process reduces the need for larger dietary intakes of protein, and in so doing reduces the energy consumption which would otherwise be needed to transport dietary amino-acids to the cell. As a consequence of this recycling, proteolytic rates are significantly underestimated (56).

Another approach which has been employed for measuring the degradation of certain proteins (such as collagen, histones, actin, and myosin) takes advantage of the unique amino-acids formed by post-translational modification. Among these are methylated arginine, hydroxyproline, and 3-methylhistidine (which will subsequently be abbreviated to 3-mehis in the remainder of this thesis)(40). As these substances are not reutilized in a further round of protein synthesis, measurement of their production

provides an accurate estimate of protein degradation. The assay of urine for hydroxyproline has, in fact, long been useful clinically to estimate collagen degradation (1, 40). An analogous method has since been developed for estimating the degradation rate of the contractile proteins, actin and myosin (55). This procedure involves measuring the 24-hour urinary output of the non-reutilizable amino-acid 3-mehis. This amino-acid originates from the intracellular hydrolysis of actin and myosin (heavy chain origin) and is excreted in the urine without taking any further part in metabolism (29, 56). Furthermore, it has been observed (51) that this substance is filtered but not appreciably reabsorbed by the kidney tubules. Therefore, an alteration in the muscular production of 3-mehis has been found to produce proportional changes in plasma concentration and urinary output (56).

However, the reliability of a urinary analysis depends on the accuracy with which the 24-hour urine sample is collected (Appendix II). In order to check the completeness of the collection, the creatinine content of several consecutive daily samples is assayed, because creatinine, which is the breakdown product of creatine (20), is excreted in a constant daily amount by any given individual (19, 50). This amount has been found to change only as a result of renal disease, fever, or severe trauma, or in response to large day-to-day fluctuations in meat consumption (24). By contrast, no such variations in daily creatinine excretion are produced by altered rates of liquid ingestion or sweating (19). Therefore, when another urinary compound is under investigation, its level of excretion is usually expressed per milligram of creatinine, rather than per urine volume. For instance, the myofibrillar protein degradation rate is considered directly proportional to the 3-mehis/creatinine molar excretion ratio (42, 56).

When the myofibrillar protein degradation rate is being estimated from the analysis of urinary 3-mehis and creatinine, it is essential that the subjects under investigation be on a meat-free diet. This dietary restriction is important because meat contains variable amounts of creatinine and 3-mehis which would find their way into urinary output and thus interfere with measurement of the amounts arising from endogenous production (50). Ward and Cooksley (50) demonstrated that subjects required at least three days on a meat-free diet before the 3-mehis and creatinine from this exogenous source could not be detected in the urine.

Recently, the specificity of the 3-mehis procedure for estimating muscle protein degradation has been questioned (22, 33). Although the body's largest source of actin (from which 3-mehis is produced) is skeletal muscle tissue, the actin of the gastro-intestinal and skin tissue is believed to turn over at a more rapid rate (22). Therefore, skin and gastro-intestinal tissue must be contributing to the urinary output of 3-mehis. Nevertheless, the estimated rates of myofibrillar proteolysis based on 3-mehis data are suggested to accord with those determined by invasive techniques (21, 31, 50, 56). This technique is widely used for in vivo human experimentation to assess the rate of muscle protein breakdown in man during disease (36, 49) and following surgery (37, 38, 52).

IN VIVO AND IN VITRO REGULATORS OF MUSCLE PROTEIN BREAKDOWN RATE

A number of in vivo factors influence the extent of muscle protein turnover. In rats, food deprivation (12), glucocorticoid-induced atrophy (12), denervation and disuse atrophy (12, 15), and genetic muscular

dystrophy (12) have all been associated with an enhanced degradation rate. On the other hand, from in vitro experimentation with rat muscle, several factors that appear important for normal growth are observed to reduce proteolytic rate: insulin, glucose, leucine, isoleucine, valine, and repeated electrical stimulation of muscle (12). These results suggest that the muscular proteolytic rates vary in response to endocrinal and nutritional influences, as well as to differing patterns of muscular activity. Furthermore, it is believed that these factors may influence proteolysis by altering lysosomal function (13), because treatment of organ cultures with lysosomal function inhibitors retards both proteolysis (15-40%) and atrophy of muscle (13).

THE EFFECTS OF EXERCISE ON THE DEGRADATION OF MYOFIBRILLAR PROTEIN

Conflicting results have been reported for the effects of exercise on myofibrillar proteolysis. A single bout of prolonged running has been variously observed to result in no significant change (7), a significant elevation (8), and a significant reduction (42) in the urinary 3-mehis excretion in the 24 hours after exercise (as compared to the 24 hours before exercise). Rennie et al. (42) also observed a significant drop in the muscular production of 3-mehis, in conjunction with the drop in urinary output, during an endurance bicycling session (as measured by a reduced 3-mehis content of muscular biopsies taken during the exercise).

The effect of exercise on the cellular system which many investigators believe controls degradation--the lysosomal system--is not conclusively determined. An increase in lysosomal enzyme activity has been observed

in the endurance-exercised muscle of untrained mice but not of trained mice (48). Vihko et al. (48) suggested that exercise somehow results in injury of the untrained muscle tissue and stimulates the lysosomal system to degrade the injured tissue. Other investigators have reported no changes in lysosomal fragility (40) and no alteration in the activity of lysosomal enzymes of rat muscle after exhausting endurance bouts of running (8, 45). However, the latter studies did not report the fitness of their rats. Therefore, without further experimental support, Vihko et al.'s (48) suggestion that untrained muscle is more susceptible than trained muscle to injury and consequent proteolysis during exhausting exercise cannot be considered conclusive.

Although little experimental attention has been focused on the effects of muscle growth stimulating exercise (such as progressive resistance training) on the degradation of myofibrillar protein, popular myth has it that a weight training session damages muscle tissue, and that a period of recovery is required to somehow restore the tissue to normal. The soreness which accompanies a particularly strenuous bout of training is believed by some coaches to be evidence of damage which causes functional deterioration. Whether this soreness is actually due to an enhanced muscular proteolysis as a result of the weight training exercise is unknown. The results of one study (8) indicate that an increase in the proteolytic rate of myofibrillar protein occurs as a consequence of a powerlifting bout, as observed by an elevated excretion of 3-mehis (urinary) in the 24-hour period after the bout. If these results accurately reflect the effect of weight training on the proteolytic system, then the growth of muscle which normally accompanies this type of training must be due to an increased synthesis rate (as the balance of synthesis and proteolysis determines

the amount of muscle stored). Furthermore, work-induced hypertrophy of chicken muscle (26) and compensatory growth of rat muscle after synergist tenotomy (16) both occur in association with an increased proteolytic rate. However, growth of atrophied soleus muscle in the rat has been linked with a decrease in proteolysis (15). In order to clear up this controversy, other studies (2, 3) have attempted to simulate *in vivo* growth in tissue cultures, which facilitate the measurement of the rates of synthesis and proteolysis. The results from these tissue culture studies have indicated that when growth-producing substances such as insulin, growth hormone, and amino-acids are added to a culture, they inhibit proteolysis (2).

As yet, there has not been any investigation reported which looks directly at the long-term effects of progressive resistance training on the degradation rate of human myofibrillar protein; but results have been reported from the investigation of the excretion of urinary nitrogen, which is an indicator of total body protein catabolic rate, in response to progressive resistance training. Urea, the metabolic product from all body proteins, makes up most of this urinary nitrogen pool. All the studies that have looked at urinary urea excretion in response to training report that there are transient increases in the output of this substance after a single bout of strenuous exercise, whereas the average urea excretion over a period of training is not increased (6, 18). Indeed, a decreased nitrogen excretion has been observed with weight training, accompanied by an overall positive nitrogen balance in the body (30). The decreased excretion of nitrogen observed in these studies indirectly supports the contention that there is a decreased breakdown of myofibrillar protein taking place as a result of training, because muscle tissue is a

large contributor (30%) to the total body protein pool (56) and thus any major changes in muscular proteolytic rate would be expected to significantly alter the total amount of nitrogen excreted. By employing the urinary analysis of 3-mehis for estimating skeletal muscle degradation, in conjunction with the measurement of urinary urea excretion, a better understanding of the contribution that proteolysis makes during muscle growth may be reached.

STATEMENT OF THE PROBLEM

Progressive overload resistance training induces growth of skeletal muscle tissue. Muscular mass is increased with this type of training, because the exercised muscle cells store larger amounts of myofibrillar (contractile) protein. However, the biochemical mechanisms responsible for the augmentation of myofibrillar protein deposition have not been fully elucidated (3, 4). Investigation has demonstrated (44), that the interaction of protein synthesis and protein degradation controls the quantity of any intracellular protein. The study of these two processes, has lead to the general conclusion that myofibrillar protein synthesis is enhanced during work-induced muscular growth, but agreement has not been reached on what happens to the proteolytic process. The aim of this investigation is to determine whether progressive resistance training does indeed affect the rate of myofibrillar proteolysis, and therefore establish the possible contribution of the degradative pathway to work-induced muscular growth.

GENERAL HYPOTHESIS

If untrained subjects participate in daily bouts of progressive resistance exercise, the myofibrillar proteolytic rate of their skeletal muscle tissue (as measured by the 24 hour urinary excretion of 3-methylhistidine) will be significantly different from that of a non-training group.

NULL HYPOTHESES

1). There is no difference between the myofibrillar proteolytic rate of a group of subjects participating in progressive resistance training and that of a non-training control group.

2). There is no difference between the myofibrillar proteolytic rate of the experimental group(training) when they are tested on six pre-selected days during the training period.

METHODS AND PROCEDURES

SUBJECTS

Sixteen males aged between 17 and 29 years acted as subjects. They were all students of the University of Windsor. Although most of these individuals were habitually active in some form of sporting program, their experience with regular resistance training was limited. The subjects were randomly organised into two groups: an experimental group ($N = 10$) and a control group ($N = 6$).

THE EXPERIMENTAL PERIOD

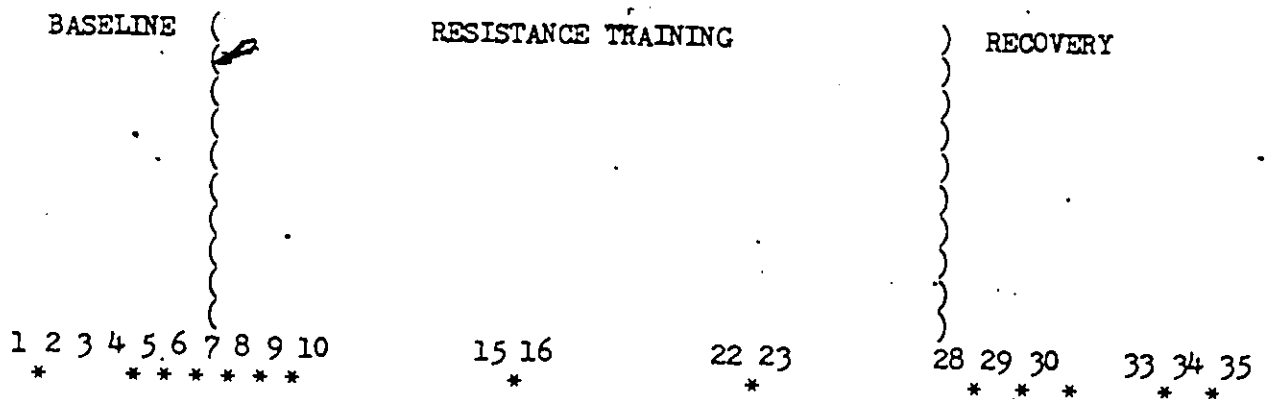


FIG. 1: The experimental period, indicating the 14 urine collection days (*).

The experimental data were compiled over a five-week period (Fig. 1). This period was made up of three distinct experimental phases: a baseline phase of one week duration, a resistance phase lasting three weeks, and a recovery phase of one week. On the first day of the experimental period, all subjects were placed on a meat-free and relatively constant protein

quantity diet. Each subject received documents which outlined alternative protein sources commonly available (Appendix I). These dietary restrictions were enforced until the completion of the experimental period. A meat-free diet was considered essential because, as previously indicated the urinary excretion of 3-mehis was used as the estimator of protein degradation in this experiment, and meat contains variable amounts of this metabolite. Although three days may be sufficient to clear the system of dietary 3-mehis (50) seven meat-free days (the baseline period) preceded the initiation of the training period in this study.

On fourteen different days (indicated by the asterisks in Fig. 1) throughout the five-week period, subjects collected 24-hour urine specimens in washed and labelled two-liter flasks (Appendix II). The specimens were received from each subject on the day after a collection. Upon receipt, the urine volume of each specimen was measured, and aliquots needed for further analysis were taken and refrigerated at -20°C .

Subjects were tested for strength (p.16) and body density (Appendix IV) prior to the first day of the baseline phase. Following this day, all subjects were asked to refrain from resistance training until the 7th day of the experimental period (1st day of the training period). On this day, the experimental group began participating in daily one hour resistance training sessions (p. 17), while the control group remained sedentary. The training phase continued for three weeks, and all the training information was recorded for each subject. A non-

training or recovery week followed the resistance training phase, during which time urine collections were continued at pre-selected intervals (Fig. 1).

THE FLUOROMETRIC ANALYSIS OF URINARY 3-METHYLHISTIDINE

Urinary 3-mehis was analysed by the following fluorometric procedure (35):

Two 0.1 ml aliquots of a diluted urine sample (1:20 aqueous dilution) were transferred to two glass test tubes (with tightly fitting caps), and 0.3 ml of 0.1M potassium tetraborate buffer (pH 9) was added to each. While the tubes were being vigorously stirred (vortex mixer), 0.1 ml of an aldehyde mixture (5% glutaraldehyde and 2.5% formaldehyde in water) was rapidly added to each. The tubes were then allowed to stand at room temperature for ten minutes. Subsequently, while the tubes were being vigorously stirred again, 1 ml of an acetonitrile solution of fluorescamine (20mg/100 ml) was rapidly added to one tube (the sample tube), and 1 ml of 2N HCL was rapidly added to the other tube (the blank tube). Both tubes were then allowed to stand at room temperature for a further ten minutes before the fluorescamine/acid adding procedure was reversed. Both tubes were tightly capped and incubated in a water bath at 70°C for one hour. After cooling, the fluorescence of both tubes was read in a Ratio-2 fluorometer fitted with a Corning 7-60 primary filter, and a Wratten 2A secondary filter, which had been zeroed with a standard blank. The fluorescence difference between the two tubes was proportional to the 3-mehis concentration. The coefficient of variation for this method is given on page 37.

ANALYSIS OF URINARY UREA-NITROGEN AND CREATININE

Urinary urea-nitrogen and creatinine were analysed with the Beckman urea-nitrogen and creatinine chemistry modules, which are sub-systems operating within the Beckman Astra 8 Automated Stat-Routine Analyser System. The urea-nitrogen module employs the conductivity rate sensing mechanism (39), while the urinary creatinine module uses a rate sensing mechanism (23).

STRENGTH TESTING AND TRAINING PROCEDURES

The Strength Testing Procedure

1. On the day prior to the baseline period, the strength of all the subjects was estimated. By trial and error, the maximum weight that each subject could use for eight repetitions (8 RM) of the ten exercises specified (table below) was determined.
2. A mean 8 RM weight was determined from the procedure outlined above, and this value was used as the strength index. The strength index was expressed in kg for all subjects.

The Resistance Training Exercises

Day 1	Day 2
Bench Press	Pullovers
Leg Press	Knee Extensions
Power Cleans	Stiff Legged Dead Lifts
Bicep Curls	Knee Flexion
Overhead Press	Bent Rowing

The Progressive Resistance Training Program

The experimental group participated in three weeks of progressive overload resistance training. The program consisted of daily sessions during which time five exercises were used. The exercises are shown in the table on page 16. These two groups of five exercises were used on alternate days throughout the training period. The subjects attempted to complete three sets with the 8RM weight, which had been determined in the pre-experimental period testing, for a given exercise each day. If three sets with the 8RM weight were accomplished, then the weight used for this exercise in two days time, was increased. The weight increase recommended was 2.5 - 5.0 kg for trunk and arm exercises and 5.0 - 10.0 kg for leg exercises. All changes in the weights were recorded on a suitably designed program chart. The subjects were shown correct lifting procedure, and their sessions were closely monitored by the experimenter.

RESULTS

The pre and post-training subject characteristics are presented in Table 1 (p. 19). The two groups were well matched with respect to strength prior to the training regime (p. 17). There was no significant difference between the strength index means for the two groups at this time. However, following three weeks of strength training, the strength index for the experimental group increased by 18% and was significantly different from the pre-training value. Pre- and post-training body weights and densities were not significantly different (for both groups).

The excretion of urinary creatinine by the experimental group was higher than that by the control throughout the experiment (Fig. 2). However, this difference was not significant on any given day, nor was there a significant difference between days for a particular group. The experimental group's higher mean body weight (74kg as compared to 70kg for the control group) may have been the factor responsible for its higher creatinine excretion. The daily variation in the creatinine excretion for the two groups was in the order of 4-8%, which was similar to the variation reported by earlier investigators (19, 50). The group creatinine excretion means were: experimental 14.80 SE. 0.79 mmol/day; and control 13.88 SE. 1.09 mmol/day. These values were within the range for normal adult males, reported by other investigators (19, 50).

Figure 3 presents the data collected for the effects of resistance training on the excretion of 3-methylhistidine (when expressed per mmol of creatinine per day). The 3-mehis/creatinine ratio for the experimental

SUBJECT CHARACTERISTICS

Group	Body Weight (KG)		Body Density (g/cc)		Strength Index (KG)	
	Initial	Final	Initial	Final	Initial	Final
control N 6	69.96	70.14	1.071	1.068	49.85	49.25
	S.E. 4.38	S.E. 4.36	S.E. .004	S.E. .004	S.E. 2.62	S.E. 2.96
experimental N 10	74.27	74.52	1.061	1.059	50.06	59.35*
	S.E. 4.98	S.E. 3.88	S.E. .005	S.E. .003	S.E. 2.62	S.E. 2.96

TABLE 1: The influence of three weeks of progressive resistance exercise on subject characteristics.

*The increase in the strength index for the experimental group was significant at the .05 level (dependent t ratio).

group was not significantly different from that of the control group during the baseline period. The baseline excretion rates for both groups were within the normal range of 16-22 $\mu\text{mol}/\text{mmol}/\text{day}$ reported for adult males (56). However, after only two days of training, the experimental group mean dropped significantly (Analysis of Variance Appendix XI) from that of the last baseline day level. In contrast, the control group's 3-methylhistidine/creatinine ratio remained close to the baseline level throughout the experimental period. Analysis of Co-variance with repeated measures (using the baseline excretion values for both groups of subjects as the co-variate), indicated that the excretion ratios for the two groups were significantly different ($F = 28.3$, $df\ 1, 83$) during the training period but not at other times during the experimental period (Appendix X). By the second day of the recovery period, the excretion ratio for the experimental group had returned to baseline level, and it therefore had increased significantly from the level on the last training day.

The data in Figure 4 present a graphic view of the influence that resistance training had on the urinary excretion of urea (expressed as $\text{mol urea}/\text{mol creatinine}/\text{day}$). While the urea/creatinine ratio for both groups was similar during the baseline period, by the second day of training the experimental group's ratio was significantly different from that of the non-training control group; it remained below baseline level for the experimental group, and was significantly different from the control group's ratio, until the second week of training.

The data presented in Figure 5 show the regression line for the pre-training body density data and urinary creatinine data. The significant positive correlation found for these two parameters ($r, .75$) concurs with the results of other studies, and indicates that the subjects were adhering to the meat-free diet.

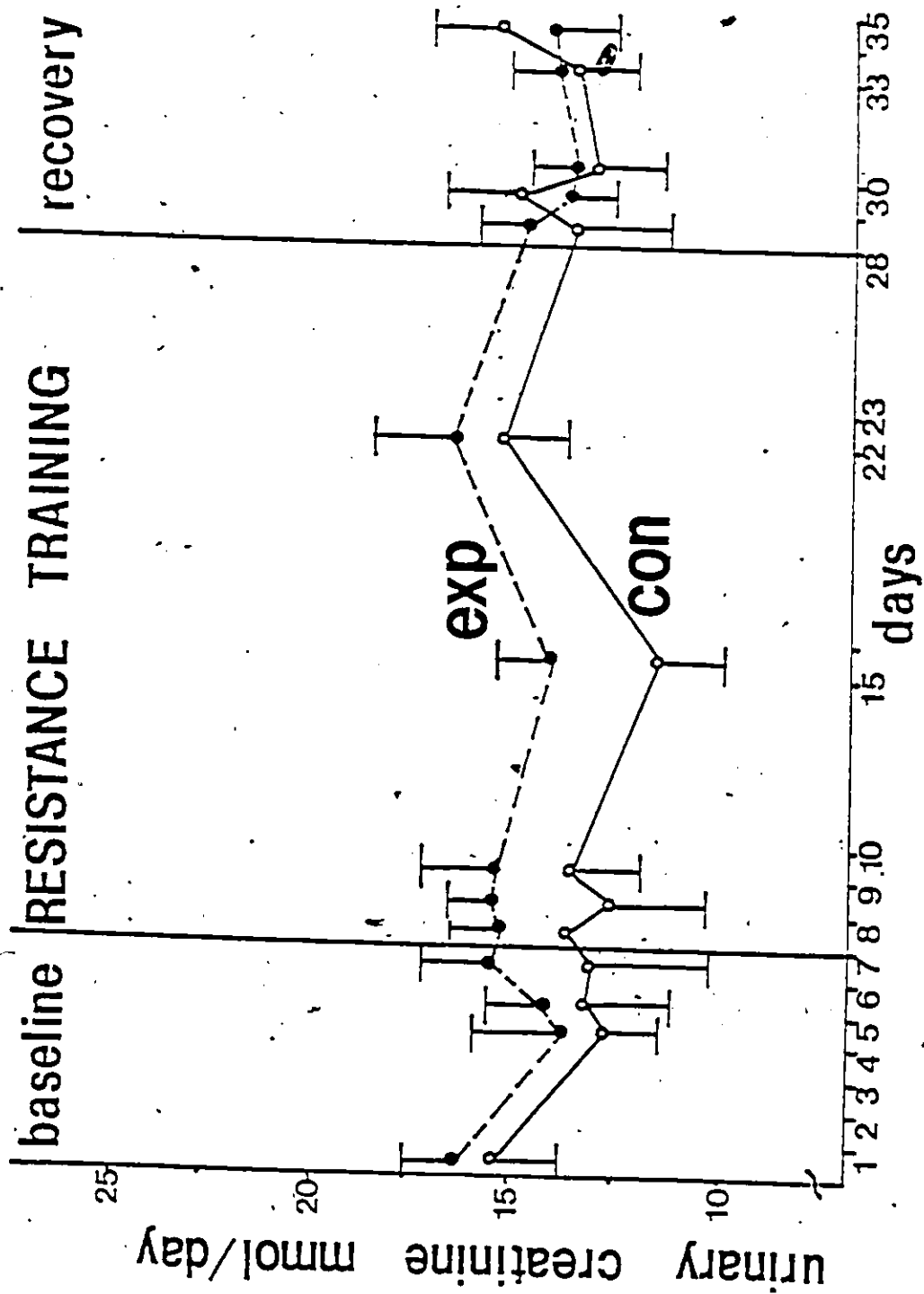


FIG. 2: The influence of progressive resistance exercise on the urinary excretion of creatinine.

The linear regression equation calculated for the two variables was:

$$\text{BODY DENSITY} = 0.963 + .0044(\text{CREATININE EXCRETION})$$

(mg/kg BW/day)

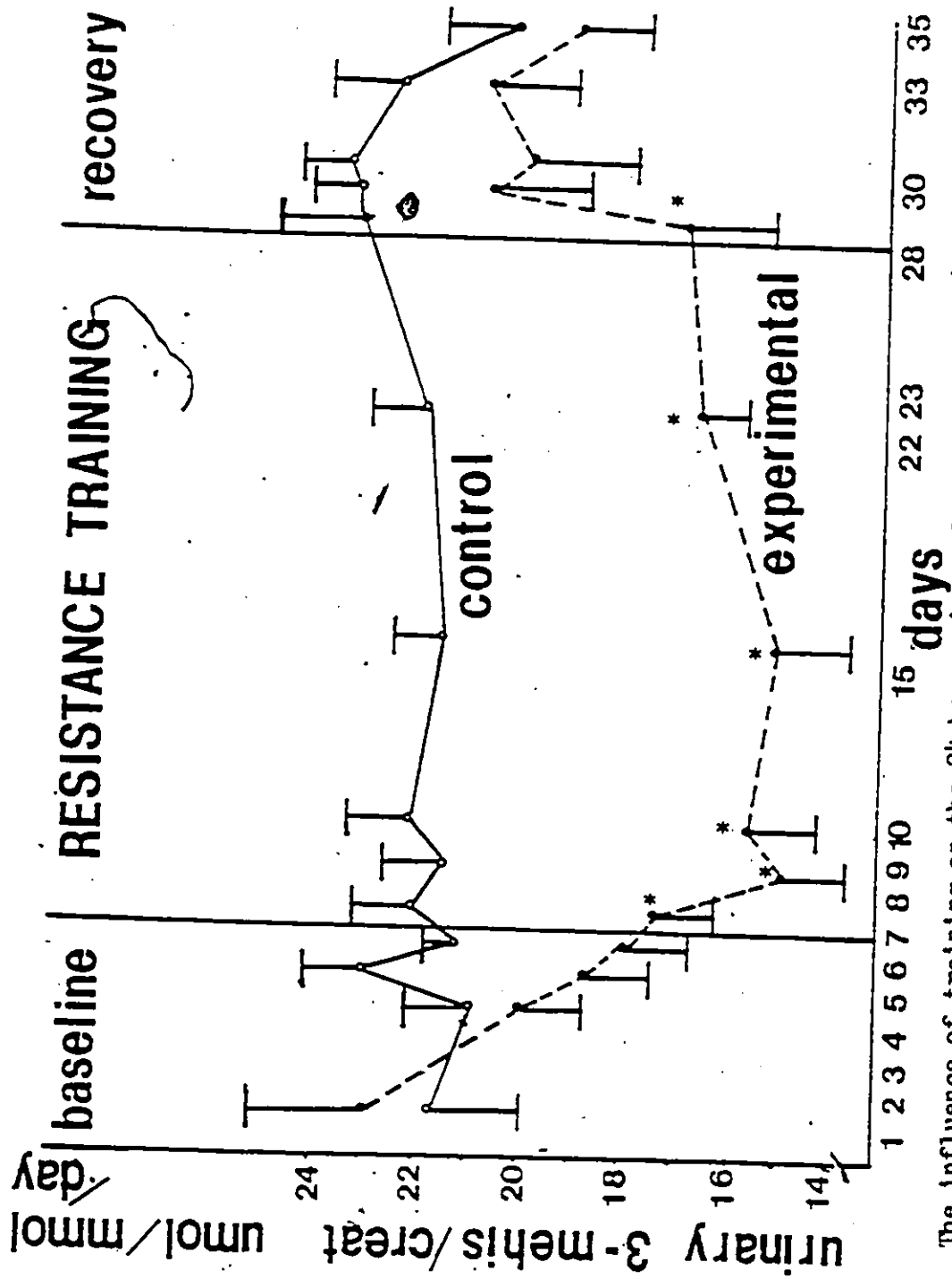


FIG. 3: The influence of training on the 24-hour urinary 3-methylhistidine/creatinine ratio.
 *Analysis of co-variance reveals a significant difference ($P < .001$) between control and experimental groups on these days.

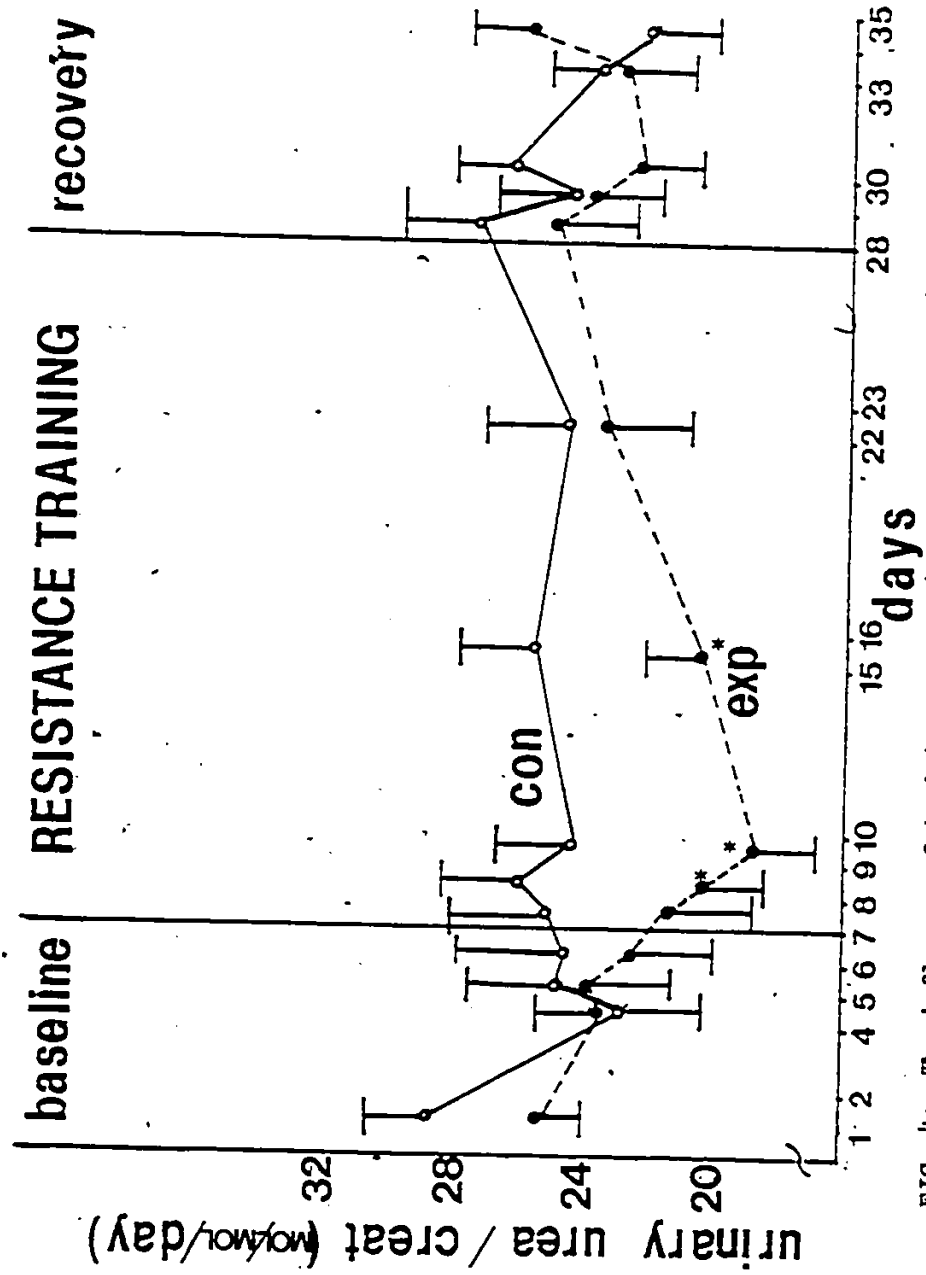
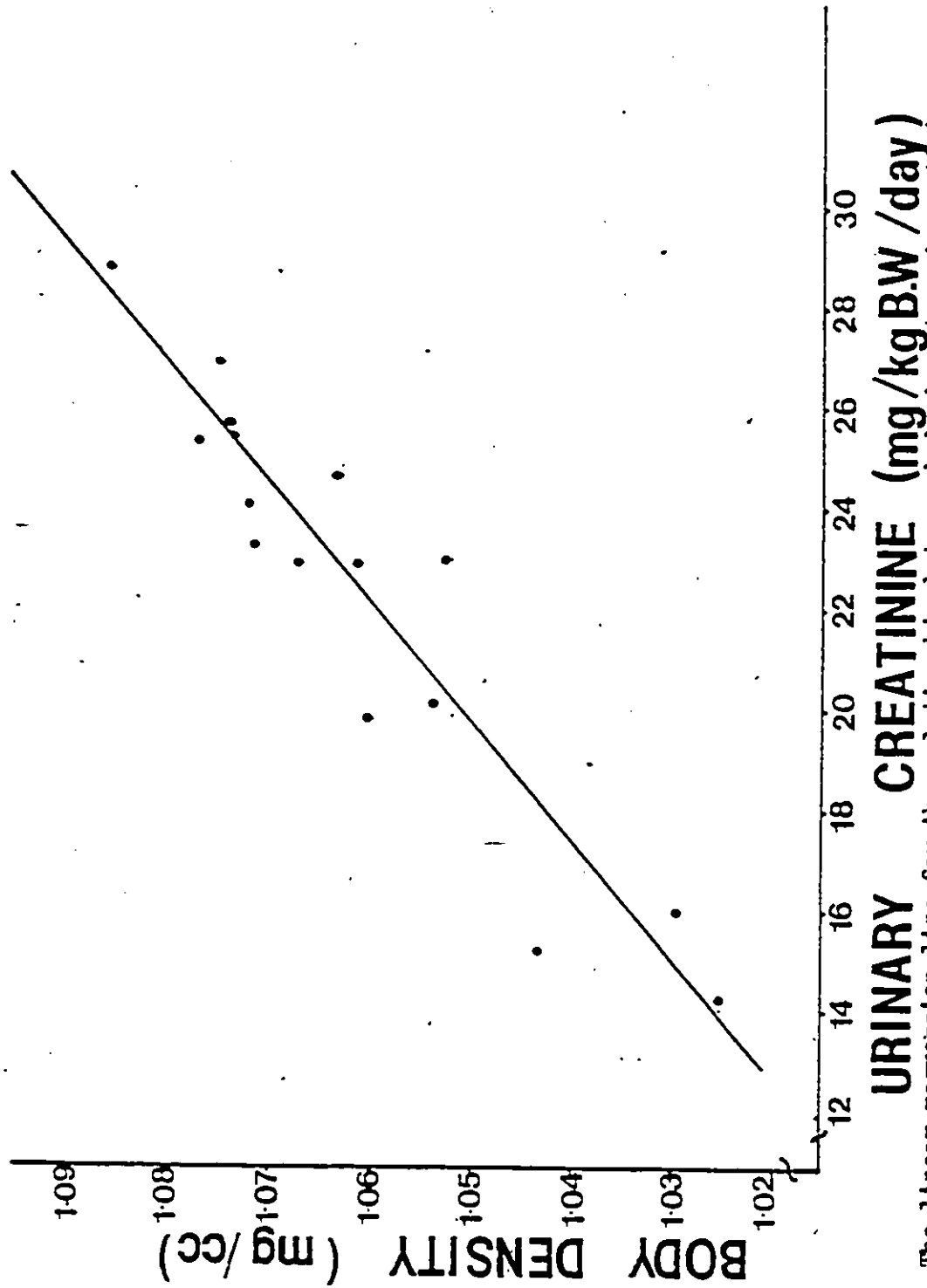


FIG. 4: The influence of training on the 24-hour urinary urea/creatinine ratio. *Analysis of co-variance reveals a significant difference ($p < .001$) between control and experimental groups on these days.



URINARY CREATININE (mg/kg B.W./day)

FIG. 5: The linear regression line for the relationship between body density and creatinine excretion. There was a significant positive correlation ($r = .75$, $P < .001$) between the two variables.

DISCUSSION

The present study indicates that a rapid reduction in the degradation rate of human myofibrillar protein occurred as a result of the resistance training program, which enhanced the strength index (p. 16) of the experimental group by 18% (Table 1). Direct evidence for this conclusion was obtained from the finding that the 3-methylhistidine/creatinine daily excretion ratio (an indicator of myofibrillar protein degradation rate) dropped by 18% (dep t-ratio, 2.33, df, 9) after 2 days of training from the level on the last baseline day (Fig. 3). The 3-mehis/creatinine ratio for the experimental group remained significantly depressed (below the baseline) throughout the first two weeks of training, and after three weeks it was still 6% below baseline. However, during the recovery period this ratio rose rapidly, indicating that a quick increase in the degradation rate occurred when the training stimulus was removed. The 3-mehis/creatinine ratio for a non-training control group did not undergo any significant changes throughout the same period. These data suggest that one mechanism contributing to the positive nitrogen balance observed by previous investigators (6, 30) during strength training, is the reduction in skeletal muscle proteolysis.

As was anticipated from earlier investigations (8, 41) urinary creatinine excretion remained stable throughout the training period (Fig. 2). This result suggests that the excretion of 24 hour urinary creatinine is unaffected by progressive resistance exercise; therefore,

the reduction in the 3-mehis/creatinine ratio that occurred in this study, can be attributed to a drop in the 3-methylhistidine excretion.

Consequently, the decrease in 3-mehis excretion observed in this study is in agreement with the results obtained by Rennie et al (42), who investigated the renal handling of 3-mehis during an endurance bicycling bout. They found that the renal clearance of 3-mehis fell during exercise by approximately 50%, but that this drop was not wholly due to the reduced renal blood flow which also took place, and suggested that some of the reduced clearance was due to a drop in the muscular production of 3-mehis during exercise. Therefore, Rennie et al took biopsies during the exercise, and they observed that the 3-mehis content of these biopsies was indeed lower than the 3-mehis content of biopsies taken before exercise. Rennie et al's results suggest that the increased muscular activity which occurs during exercise is somehow affecting a reduction in myofibrillar proteolysis.

In contrast to the results of Rennie et al (42), the results of Dohm et al (8) demonstrated that a significant increase in the excretion of 3-mehis occurred after a single powerlifting bout ($N = 4$). However, there are differences between the subjects used by Dohm et al (8) and those used by both Rennie et al (42) and by the present investigators, which may account for this seemingly discrepant result. Firstly, the subjects in the present investigation were untrained, while those in Dohm et al's investigation were experienced powerlifters. Secondly, the subjects in the present study excreted 3-mehis at the rate of $3.7 \mu\text{mol/kg/day}$, which is similar to the value for normal adult males of $3.2 \mu\text{mol/kg/day}$.

(42, 56), while the powerlifters used in Dohm et al's study had an excretion rate of more than twice this magnitude, 6.5 $\mu\text{mol/kg/day}$. This would suggest that constant training of muscle may affect the way in which its proteolytic system responds to exercise stimuli.

In the present investigation, supportive evidence for a reduced excretion of myofibrillar nitrogen was provided by the observation of a 7% drop in the urinary urea/creatinine ratio (an indicator of whole body protein catabolic rate). This 7% drop in the urea/creatinine ratio occurred at the same time as the 18% reduction in the 3-methylhistidine/creatinine ratio. As investigators have demonstrated that skeletal muscle contributes approximately 30% of the total urea excreted (42, 56) then the 18% reduction in myofibrillar protein breakdown (observed in the present study) could account for most of the 7% drop in urea output. If it is assumed that the 3-mehis concentration of adult muscle, determined by Young and Munro (56) as 4.2 $\mu\text{mol/gm}$ of mixed muscle protein, is similar to that for the subjects used in the present study; then the average decline in 3-mehis output of 10% (28 $\mu\text{mol/day}$) over the three weeks of training would result in a conservation of an extra 140g of mixed muscle protein. A proportional increase in muscle water content would be required to store this extra protein within muscle tissue as muscle is composed of approximately 20% protein and 75% water. Therefore the total increase in muscle weight should be in the order of 700g. The present studies' data indicate that the weight increase for the trained individuals was 250g. Although this is less than predicted, given that the values for the 3-mehis concentration of muscle and the percentage water

content used in the predictive calculations are approximate, it is acceptable. In addition this finding concurs with Wilmore's (53, 54) observation that for the average college age male a ten week weight training program resulted in little or no change in total body weight.

Although until now there have been no reported studies of the effects of long term strength training on the excretion of 3-mehis, there have been several on its effects upon the excretion of urinary nitrogen (6, 18, 30). One conclusion common to all these was that there were transient increases in nitrogen excretion after a bout of strenuous exercise (as in a prolonged competitive event), whereas the average nitrogen excretion over a period of strength training was not increased. Indeed, during some strength training programs a decreased urinary nitrogen excretion has been observed (30), in conjunction with an overall positive nitrogen balance in the body. As previously suggested, the present investigation also found that a decreased excretion of nitrogen occurred with training. Furthermore, the present study provided evidence which indicated that this decrease in nitrogen excretion might have been due to a reduced skeletal muscle proteolytic rate.

The possible regulators of muscular proteolytic rate have been studied (2) in tissue cultures. It was suggested that proteolysis is regulated by the activity of the intracellular lysosomal system. In addition, several substances such as insulin, growth hormone and amino-acid perfusion have been proposed as controllers of in vivo proteolysis (3). Therefore, the reduced proteolysis observed in the present study may have occurred as a result of, 1) a direct inhibitory action of the muscular activity on the lysosomal system, 2) a post-exercise increase in insulin secretion, 3) an enhanced perfusion of muscle with amino-acids, or 4) a combination of these factors. The observed speed of this response may provide the organism

with a rapid means by which it can conserve protein. This ability of the proteolytic system to respond rapidly, may be advantageous to the organism, as alterations in the protein synthetic process (which is the other protein conserving mechanism available), may take longer to occur (11, 28, 55). Indeed, when growth of rat myoblast tissue occurred in vitro, investigators observed (13) that proteolysis decreased (15-40%), but that protein synthesis rate remained unchanged after a short incubatory period. The complexity of the synthetic pathway may be higher than that of the degradative pathway, and therefore more time is needed to change its rate in response to stimuli. Furthermore, when protein synthesis is enhanced after a long period of training (11, 57), proteolysis may not need to be inhibited to the same extent. The latter suggestion may be supported by the observation of increases in the 3-mehis/creatinine ratios (in the present study) after two weeks of resistance training.

CONCLUSIONS

In conclusion, this study has demonstrated that progressive resistance training reduces the myofibrillar proteolytic rate of skeletal muscle. Hence, this study suggests that a process of protein conservation takes place as a result of the decreased myofibrillar proteolytic rate produced by the resistance training stimuli. This conservatory mechanism seems to respond rapidly to a resistance training stimulus, as only two days were required before a significant reduction in the proteolytic rate took place; and when the training stimulus was removed it took only two days before the proteolytic rate returned to pre-training levels, indicating that protein was no longer being conserved by this mechanism. As a corollary to the main investigation, this study confirmed the results of other investigators who suggested that strength training has little effect on the 24 hour urinary excretion of creatinine.

APPENDIX I

VEGETARIAN FOOD GROUPS AND SERVING SIZES

Food Group	Serving Size
Milk and calcium equivalents	
Milk	1.00 cup
Yogurt	1.00 cup
Cheese, natural	1.50 ounces
Ice cream	1.75 cups
Cottage cheese	2.00 cups
Soy milk, calcium fortified	1.00 cup
Broccoli, collards	1.00 cup
Turnip greens	1.25 cups
Kale, mustard greens	1.50 cups
Beet greens	2.00 cups
Meat alternatives and protein equivalents	
Eggs, small	2
Cheese, natural	1.50 ounces
Cottage cheese	0.50 cup
Milk	1.33 cups
Soybeans, cooked	0.50 cup
Tofu (soybean curd)	4.00 ounces
Meat analog	various
Textured vegetable protein	various
Peanut butter	2.50 tablespoons
Peanuts	0.33 cup
Split peas, common dried	
bean varieties, lentils	0.66 cup
Black-eyed peas, cowpeas	0.75 cup
Almonds, cashews	0.50 cup
Walnuts, filberts	0.66 cup
Pecans	1.00 cup
Sunflower seeds	0.50 cup
Sesame seeds	0.66 cup
All of the above food portions contain the protein equivalent of 1 ounce of meat or 7-8 grams of protein. Other foods recommended for the lacto-ovo vegetarian include the following:	
Fruits and vegetables	
Cooked or juice	0.50 cup
Raw	1.00 cup, or 1

	piece or portion
Grains	
Bread	1 slice
Cereal, ready to eat	1.00 cup
Cereal, cooked	0.50 cup
Pasta, grits, rice	0.50 cup

RECOMMENDED MINIMUM DAILY SERVINGS AND PROTEIN

CONTRIBUTION OF VEGETARIAN FOOD GROUPS

Food Group	Minimum Daily Servings		
	Child	Teenager	70-kg Adult
Milk and calcium equivalents (protein, g)	3-4 (24-32)	4-5 (32-40)	2 (16)
Meat alternates (protein, g)	2 (20)	3 (30)	2-3 (20-30)
Fruits and vegetables (protein, g)	4 (8)	4 (8)	4 (8)
Grains	4 (8)	4 (8)	4 (8)
TOTAL PROTEIN, g	60-68	78-86	52-62

Courtesy, National Dairy Council, Vegetarian Nutrition

APPENDIX II

THE 24-HOUR URINE COLLECTION PROCEDURE

All subjects were supplied with 2-litre flasks in which urinary collections were made on the days specified on a calendar provided. The following procedure was carefully explained to each subject prior to the experiment, and a copy of these instructions was made available.

1. The subject will empty the bladder at a specified time on the night before a collection. This time must be recorded on the label of the collection container. The urine on this first urination must be discarded.

2. The subject will then collect all urine voided during the next 24 hours.


3. The last specimen will be collected at the same hour as the first discard, on the following day. The subject should then put his name and the following details on the container label, i.e.:

J. Doe began collection 8:00 pm Nov. 10
finished collection 8:00 pm Nov. 11

4. The subject was directed to take the urine container with him to all places during a collection period.

APPENDIX III

INFORMED CONSENT FOR PARTICIPATION IN THE
TRAINING/URINARY ANALYSIS STUDY

1. Explanation of the training: You will perform a weight training regime; of a progressive resistance overload nature. The exercise intensities will be at a level that you can accomplish without suffering injury.
 2. Explanation of the diet: You will be required to remove meat from your dietary intake for a period of five weeks, beginning at 12 midnight November 10/1982 and ending December 15/1982. You will not be paid if meat breakdown products are detected in your urine sample. If you drop out of the experiment prior to its completion, you will not receive any money.
 3. Urinary Collection: You will be required to collect 24 hour urine samples according to the procedure outlined in the hand-out, and at the times specified on your program calendar.
 4. Freedom of consent: Your permission to perform this program of exercise, in addition to adhering to a meatless diet and urine collection, is voluntary. You are free to deny consent if you so desire. I have read this form and I understand the test procedures that I will perform. I consent to participate in this program.
- Date: Signature of subject: 

Witness:

APPENDIX IV

PROCEDURE FOR DETERMINING BODY DENSITY

Weight in Air (W_a) = _____ gm

Room Temperature = _____ °C BTPS Correction = _____

Vital Capacity (VC) = _____ mls

Residual Volume (RV) = 0.24(VC) mls

Temperature of Water in Tank = _____ °C

Density of Water at this Temperature (D_w) = _____ gm/ml

Weight of Subject in Water (W_w) = _____ gm

DENSITY OF THE SUBJECT'S BODY (D_b , gm/ml):

$$D_b = \frac{W_a}{\frac{W_a - W_w}{D_w} - RV}$$

This method was obtained from; Goldman, R.F., and Buskirk, E.R. "Body Volume Measurement by Underwater Weighing: Description of a method," in Brozek, J., and Henschel, A., (Eds), Techniques for Measuring Body Composition, Washington, D.C.: National Academy of Science, 1961.

APPENDIX V-A

COEFFICIENT OF VARIATION FOR 3-METHYLHISTIDINE ANALYSIS

No. of Run	Value for Standard (umol/day)
1	378
2	311
3	311
4	311
5	365
6	298
7	392
8	284
9	338
10	270
11	252
12	257
13	320
14	337
15	294
16	294
17	302
18	364
19	364
20	378
21	297
22	358
23	326
24	294
25	336
26	294
MEAN	320.39 umol
S.D.	37.62

$$CV \text{ for 3-Mehis} = 37.62/320.39 \times 100$$

$$= 11.74\%$$

Recovery Experiments

Urine Control	•Fluorescence Added Standard	Theoretical Urine +Stand	Obtain	% Recovery.
10.5	25.0	35.5	33.00	93
12.5	24.0	36.5	36.50	100
10.5	14.0	24.5	22.10	90
12.5	15.0	27.5	24.18	88
13.5	14.5	28.0	26.00	93
11.0	14.5	25.5	23.40	92
14.0	14.5	28.5	25.50	90
8.5	11.0	19.5	19.50	100

MEAN RECOVERY = 93%

S.D. = 4

APPENDIX V-B

COEFFICIENT OF VARIATION FOR CREATININE AND UREA ANALYSIS

No. of Run	Value for Standard	
	Creatinine (mg/dl)	Urea (mg/dl)
1	104	36
2	103	36
3	103	36
4	103	36
5	103	36
6	104	35
7	104	35
8	105	38
9	105	38
10	105	38
11	105	40
12	104	37
13	106	36
14	103	34
MEAN	104.07	36.5
S.E.	0.96	1.5

CV for creatinine = $0.96/104.07$
= 0.9%

CV for urea = $1.5/36.5 \times 100$
= 4.1%

APPENDIX VI

RAW DATA: SUBJECT CHARACTERISTICS

Group	Subject Number	Body Weight (KG)		Body Density (g/cc)		Strength Index (KG)		
		Initial	Final	Initial	Final	Initial	Final	
control (N=6)	1	69.55	69.32	1.069	1.066	48.63	47.50	
	2	72.27	72.50	1.053	1.050	54.09	52.50	
	3	52.50	52.21	1.083	1.082	27.73	26.50	
	4	64.09	65.45	1.077	1.069	38.41	41.25	
	5	79.54	79.54	1.076	1.075	77.05	75.00	
	6	81.81	81.80	1.067	1.067	53.18	53.00	
	MEAN	69.96	70.14	1.071	1.068	49.85	49.29	
	S.D.	10.74	10.74	.011	.011	15.22	14.56	
experimental (N=10)	7	61.36	62.27	1.066	1.058	37.27	40.35	
	8	62.27	63.63	1.073	1.069	43.18	55.60	
	9	55.45	55.86	1.058	1.058	45.45	51.36	
	10	96.36	99.09	1.030	1.030	48.18	57.18	
	11	80.91	79.09	1.054	1.056	45.45	55.23	
	12	81.36	80.45	1.073	1.073	53.64	60.75	
	13	71.82	71.81	1.074	1.070	66.82	76.36	
	14	81.82	80.90	1.042	1.037	59.09	63.84	
	15	79.55	80.45	1.074	1.075	56.05	64.92	
	16	71.82	72.50	1.067	1.065	45.45	67.95	
		MEAN	74.27	74.52	1.061	1.059	50.06	59.35
		S.D.	12.20	12.26	.015	.011	8.30	9.36

APPENDIX VII

RAW DATA: THE URINARY EXCRETION OF CREATININE (mmol/day)

Collection Subject No. and Group		Baseline				Resistance Training						Recovery				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Control	{	1	18.99	11.48	15.67	18.72	16.52	17.40	18.78	12.26	17.42	17.05	10.32	11.52	16.25	16.21
		2	15.99	14.48	12.80	17.67	12.55	14.06	14.31	9.89	14.87	12.39	13.89	16.51	11.51	14.73
		3	12.17	14.03	12.53	3.76	4.62	6.02	5.00	4.67	10.25	8.62	14.03	9.93	10.00	10.00
		4	9.78	8.61	6.15	5.02	13.66	6.88	8.77	7.95	13.12	10.80	14.73	10.85	14.50	18.90
		5	19.88	15.90	19.62	18.65	19.05	19.25	18.96	19.45	18.68	18.70	18.57	18.78	17.56	20.32
		6	14.86	12.84	13.45	16.04	15.91	13.13	16.04	16.29	17.30	16.00	21.49	12.86	14.26	15.14
		MEAN				13.72	12.79	13.64	11.75	14.87	13.93	15.51	13.41	14.01	15.88	
		S.E.				1.87	2.01	2.10	2.03	1.23	1.47	1.47	1.30	1.06	1.35	
Experimental	{	7	15.05	15.79	12.50	11.74	16.79	18.99	9.06	13.78	14.51	14.61	12.60	12.15	16.07	14.66
		8	11.64	12.89	16.73	10.37	16.38	17.44	17.80	16.17	16.52	16.14	12.20	16.16	15.29	15.86
		9	12.45	12.45	11.44	10.36	10.73	12.52	12.32	11.51	12.03	12.36	12.46	12.93	11.99	13.32
		10	21.30	11.62	12.06	23.39	12.39	10.94	24.27	8.28	27.50	11.19	11.19	11.14	11.52	8.17
		11	16.66	19.20	18.98	17.57	18.11	18.60	13.61	18.31	17.97	16.61	16.52	11.65	17.20	16.27
		12	8.64	3.86	14.27	18.06	18.64	15.85	8.02	9.68	9.00	15.22	14.84	15.71	15.00	15.80
		13	16.00	16.77	15.95	16.99	10.65	12.90	20.21	15.42	16.19	16.95	16.24	16.40	14.53	16.11
		14	20.58	10.80	17.99	11.36	13.48	14.29	14.00	8.17	14.96	10.15	15.85	6.31	10.00	10.77
		15	21.51	19.97	15.77	20.25	19.16	18.67	19.39	21.64	18.59	19.00	12.63	20.98	17.50	18.44
		16	17.10	16.44	4.91	16.06	15.99	15.16	16.00	18.29	16.81	16.20	16.83	16.46	16.05	17.26
		MEAN				15.23	15.54	15.41	14.13	16.41	14.84	14.14	13.99	14.52	14.67	
		S.E.				0.96	0.86	1.61	1.79	1.45	0.84	0.64	1.21	0.98	0.94	

APPENDIX VIII

RAW DATA: THE 3-METHYLIHISTIDINE/CREATININE URINARY EXCRETION RATIO ($\mu\text{mol}/\text{mmol}/\text{day}$)

Subject No. and Group.	Baseline				Resistance Training						Recovery				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Control	1	25.22	23.71	23.56	22.62	23.77	22.50	24.86	22.30	21.81	23.01	22.73	20.50	20.09	20.68
	2	27.00	13.51	17.83	20.63	24.63	19.16	17.58	19.11	18.49	18.94	20.92	21.21	21.04	19.44
	3	23.93	22.52	25.00	20.82	25.19	26.26	26.30	26.13	23.31	30.66	27.51	26.25	26.50	26.20
	4	22.05	17.55	25.28	20.60	18.00	21.23	22.60	23.44	22.41	19.28	24.52	24.90	22.50	17.11
	5	16.46	20.50	23.25	21.43	20.02	18.04	19.46	20.60	19.21	22.15	21.35	24.60	19.33	17.95
	6	14.90	27.41	22.89	20.86	20.59	21.07	21.61	17.02	26.53	24.13	21.29	22.40	24.83	19.06
MEAN		21.60	20.87	22.97	21.16	22.03	21.38	22.07	21.43	21.96	23.02	23.05	23.31	22.38	20.07
S.E.		1.99	1.33	1.09	0.49	1.18	1.17	1.33	1.32	1.19	1.74	1.05	0.93	1.14	1.32
Experimental	7	20.52	22.49	21.38	22.16	22.16	16.65	20.61	13.82	19.36	15.76	18.18	17.87	14.93	14.46
	8	36.41	26.22	26.22	24.25	18.98	22.21	25.60	17.80	18.22	28.93	29.31	29.24	23.98	20.01
	9	18.03	19.62	21.70	17.96	18.57	16.72	14.91	22.45	19.08	23.10	22.52	19.20	22.11	19.70
	10	13.92	15.81	11.69	10.70	12.11	7.55	10.22	10.44	10.59	13.76	12.23	11.22	10.33	13.35
	11	19.42	13.14	13.29	13.14	12.94	14.17	17.51	11.16	17.72	12.99	24.55	24.40	23.22	15.53
	12	32.60	20.62	18.18	18.71	21.24	18.50	16.50	18.57	17.57	18.54	19.24	15.15	17.60	17.05
	13	16.20	14.80	13.83	17.80	18.09	12.90	12.55	11.37	13.51	11.96	14.13	18.77	23.88	26.27
	14	26.75	25.04	21.00	21.47	18.00	16.09	13.75	20.19	17.66	12.77	26.11	26.52	25.00	21.52
	15	20.43	17.06	20.13	15.22	13.92	14.10	13.00	10.54	15.58	15.09	16.92	11.80	17.05	18.25
	16	24.70	23.70	18.71	18.28	16.39	8.91	11.41	13.94	16.62	16.46	22.04	23.70	27.04	22.88
MEAN		22.89	19.85	18.69	17.97	17.24	14.78	15.61	15.03	16.59	16.94	20.52	19.79	20.51	18.90
S.E.		2.28	1.36	1.35	1.31	1.38	1.37	1.48	1.39	0.86	1.68	1.70	1.93	1.65	1.27

APPENDIX IX

RAW DATA: THE URINARY EXCRETION OF UREA-NITROGEN (g/day)

Subject No. and Group	Baseline				Resistance Training						Recovery			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	15.42	6.67	10.19	10.15	11.23	10.96	9.48	10.03	10.78	10.80	7.59	8.13	8.45	7.28
	12.54	11.41	9.23	12.00	7.35	10.15	7.94	5.92	9.41	9.43	10.15	11.34	8.35	9.54
	10.91	11.06	10.42	2.95	3.78	4.97	4.60	3.53	8.95	8.18	9.59	7.56	8.00	8.70
	6.55	2.86	2.94	2.32	4.82	2.90	4.50	3.99	3.86	6.16	8.10	6.00	8.21	7.70
	14.50	10.24	14.50	14.03	15.04	15.10	14.25	14.80	13.27	13.80	14.45	15.96	11.08	14.70
	9.52	6.51	6.30	10.56	11.28	9.36	10.32	10.40	13.16	12.00	10.61	9.47	8.20	7.90
MEAN	11.57	8.13	8.93	8.67	8.92	8.91	8.52	8.11	9.91	10.06	10.08	9.74	8.72	9.30
S.E.	1.24	1.26	1.46	1.82	1.47	1.63	1.36	1.64	1.29	1.02	0.91	1.32	0.44	1.03
Experimental	11.76	10.09	11.00	7.08	11.69	9.96	3.80	6.24	7.57	9.32	9.10	6.16	9.09	6.45
	8.10	8.97	11.21	7.61	7.45	11.48	9.02	9.18	6.32	10.19	8.70	10.80	11.84	10.88
	8.62	11.77	8.10	6.96	9.86	7.73	9.18	9.46	14.96	13.45	12.54	11.23	8.89	9.12
	13.91	6.66	6.77	13.39	8.43	5.85	15.10	5.40	16.10	6.32	6.32	6.80	4.88	6.32
	9.69	8.73	11.33	9.07	6.76	9.75	5.72	9.18	8.34	12.24	7.45	4.56	8.28	10.61
	6.44	2.11	8.38	9.10	8.19	7.06	4.28	5.85	7.51	11.35	9.20	6.80	7.00	11.08
	8.00	8.19	8.36	9.86	5.42	7.73	10.12	6.82	6.03	8.96	10.82	10.21	9.18	10.49
	12.40	4.77	8.71	5.83	6.21	4.00	6.50	3.84	9.12	5.36	8.00	4.10	5.00	7.78
	15.02	12.50	8.94	12.92	9.15	10.61	10.25	10.86	12.57	12.00	7.41	12.10	13.82	15.28
	12.27	11.62	4.37	9.58	11.21	7.94	8.05	10.16	9.40	11.45	12.00	11.88	12.04	15.50
MEAN	10.62	8.54	8.72	9.14	8.44	8.21	8.20	7.43	9.79	10.06	9.15	8.46	9.00	10.35
S.E.	0.85	0.99	0.65	0.74	0.62	0.69	1.01	0.69	1.06	0.78	0.62	1.20	1.24	0.96

APPENDIX X

ANALYSIS OF CO-VARIANCE FOR THE 24-HOUR URINARY

3-METHYLHISTIDINE/CREATININE DATA

Independent Variables (2): 1. G = Group, i.e. experimental and control
2. T = training duration, i.e. 1, 2, 3, 7, 14, 21 days

Co-Variate: the pre-training (baseline) 24-hour urinary 3-Methylhistidine/creatinine level

Dependent Variable: the 24-hour 3-Methylhistidine/creatinine data

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Probability	Beta Estimates
Mean	130.35620	1	130.35620	11.92	0.001	
G	309.62256	1	309.62256	28.30	0.000	
T	42.79346	5	8.55869	0.78	0.565	
GT	9.54150	5	1.90830	0.17	0.971	
1st Covar.	381.72363	1	381.72363	34.89	0.000	0.62668
Error	907.97192	83	10.93942			

APPENDIX XI

ANALYSIS OF VARIANCE FOR THE 24-HOUR URINARY

3-METHYLIHISTIDINE/CREATININE DATA

Independent Variables (2): 1. G is GROUP, i.e. experimental and control
 2. T is TIME DURING THE EXPERIMENTAL PERIOD
 i.e. 1, 1.....5, 6, 7, 8, 9, 10, 15, 22, 29, 30, 31, 34, 35.

Dependent Variable: The 24-hour 3-Methylhistidine/Creatinine urinary excretion data

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	TAIL PROB
G	693.76758	1	693.76758	53.13	0.000
T	209.09741	13	16.08442	1.23	0.25
GT	234.16064	13	18.01236	1.38	0.18
ERROR	2556.63940	196	13.04408		

Post-hoc analysis (Newman-Keuls) indicated that for the experimental group, the excretion ratios on days 8, 9, 10, 15, 22, 29 differed significantly from the excretion ratio on day 6 (last baseline day).

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